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Adenosine Receptor Mechanisms Underlying Bladder Dysfunction in Male Rats With Bladder Outlet Obstruction

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ABSTRACT

Aims: We examined the role of subtypes of adenosine receptors in bladder dysfunction and changes in the adenosine receptor expression in the bladder using male rats with partial bladder outlet obstruction (BOO).

Methods: In Sprague-Dawley rats (male 8-weeks old), BOO was produced by a partial ligation of the urethra along a metal rod of a 1.2 mm outer diameter. Control rats underwent sham operation. Awake cystometrograms (CMG) were first recorded during saline instillation, and then an adenosine A1 receptor agonist (CCPA, 4.1 μM), an adenosine A2A antagonist (ZM241385, 15 μM), or inosine (1 mM) were applied intravesically in sham and BOO rats. In addition, mRNA levels of adenosine receptor subtypes in the bladder wall were measured using RT-PCR. Histological studies of bladder specimen were also performed.

Results: Weights of BOO bladders were significantly ($p < 0.0001$) larger compared with sham bladders. In CMG, a number of non-voiding contractions (NVCs), bladder contraction amplitudes during voiding, bladder capacity, and post-void residual (PVR) were significantly ($p < 0.001$) increased compared with sham rats. Voiding efficiency (VE) was significantly ($p < 0.001$) reduced in BOO versus sham rats. Intravesical application of CCPA or inosine did not induce statistically significant effects on CMG parameters in BOO rats. Yet, ZM241385 induced a significant ($p = 0.040$) reduction in NVCs of BOO rats. mRNA levels of adenosine A2A and A3 receptors were significantly ($p < 0.0001$ and $p = 0.0145$, respectively) upregulated in the BOO bladder mucosa, whereas adenosine A2B receptors showed a significant ($p < 0.0001$) reduction in the BOO bladder mucosa compared with sham bladders. Histologically, we found the thickened detrusor muscle layer in BOO versus sham rats.

Conclusions: The male rat model of BOO seems to be suitable for exploring urethral obstruction-related bladder dysfunction at the compensated phase. In addition, the adenosine A2A receptor subtype would be a potential target for the treatment of male BOO patients with bladder overactivity.

Clinical Trial Registration: A clinical trial registration is not required as this study reported the basic research data using animal models.

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1 | Introduction

Both voiding and storage urinary tract symptoms are often found in elderly men with bladder outlet obstruction (BOO) due to benign prostatic hyperplasia (BPH) [1], and BPH-related storage symptoms are often resistant to treatments [2]. Thus, there is a great demand for effective treatments based on underlying pathophysiology. Also, because previous BOO research mainly utilized female animal models [3, 4], the pathophysiological mechanisms underlying male storage symptoms with BOO need further to be explored using male BOO model animals.

Adenosine produced through breakdown of adenosine triphosphate (ATP) is known to binds to the P1 class, G-protein-coupled receptor subclassified into A1, A2A, A2B and A3 receptor subtypes. It has been shown that treatments with adenosine A2A receptor antagonists [5] or A2B receptor agonists such as inosine alter bladder function [6] in female rats. However, it is not well clarified whether altered adenosine receptor mechanisms are involved in bladder dysfunction after BOO.

Therefore, we examined the alterations in bladder adenosine receptor expressions and the effects of adenosine receptor-acting drugs on bladder function in male BOO rats to explore the possibility that adenosine receptors could be a therapeutic target for the treatment of male storage symptoms associated with BOO.

2 | Materials and Methods

2.1 | Animal Model

Eight-week-old male Sprague-Dawley (SD) rats (260–288 g) were used in this study. All experiments were conducted in accordance with National Institutes of Health guidelines and the ARRIVE 10 guidelines and were approved by the University of Pittsburgh IACUC (#22122280). Rats were maintained with a 12/12 h reversed light-dark cycle and had free access to water and food in soft bedding cages. We performed BOO surgeries according to previous reports with modifications [7]. In brief, after the lower abdominal incision, the urethra was exposed and ligated between the bladder neck and the urethral fenestration using a 4-0 silk thread along with a metal rod of a 1.2 mm outer diameter, which was placed outside the urethra. Thereafter, the metal rod was removed to produce partial urethral obstruction. Sham rats had the similar procedure without urethral obstruction. After the surgery, an analgesic (buprenorphine, 0.05 mg/kg, s.c.) twice a day for 3 days and an antibiotic (ampicillin, 100 mg/kg, i.m.) once a day daily for 7 days were given. In total, 38 BOO rats and 32 age-matched sham rats were used in this study. In 30 out of 38 BOO rats and 24 out of 32 sham rats, awake, continuous cystometrograms (CMG) were obtained with or without intravesical drug administration, and in the remaining eight BOO rats and eight sham rats, histological and molecular studies were performed.

2.2 | Baseline Awake Cystometrogram

The functional experiments were performed at 4 weeks after BOO operation as shown in previous studies [7]. Under isoflurane anesthesia, rats underwent a lower abdominal incision to expose the bladder. A polyethylene tube (PE-50, Clay-Adams, Parsippany, NJ) was inserted into the bladder through the dome, and a secured suture was placed around the tube. We then placed the rats in restraining cages (Yamanaka Chemical Ind. Ltd, Osaka, Japan; 150 mm height x 80 mm width × 300 mm length). Thereafter, rats were fully recovered from anesthesia, and cystometrograms were recorded through the bladder catheter that was connected to a pressure transducer by a three-way stopcock. According to previous reports, we initially set the infusion rate at 10 mL/h, and then adjusted it to 2–20 mL/h to maintain intercontraction intervals around 20 min, because of variability in bladder capacity that is usually observed in BOO model rats [8]. Following the initial stabilization period for at least 60 min, the averaged value of each CMG parameter was calculated from measurements of 3–5 voiding cycles during 60–70 min of CMG recordings. The following CMG parameters were examined; (1) the time between reflex bladder contractions as "intercontraction intervals (ICI)," (2) the peak pressure minus the basal pressure during each contraction period as "amplitudes," (3) the pressure immediately after voiding bladder contraction as "baseline pressure," and (4) the pressure immediately before voiding bladder contraction as "threshold pressure." Also, we measured small-amplitude bladder contractions that occur without voiding with pressure elevation > 4 cm H_2O from baseline pressure during bladder filling as "non-voiding contractions (NVCs)" according to previous reports [8]. We collected voided urine to measure voided volume, and measured PVR that was obtained through the intravesical catheter by gravity immediately after voiding. The sum of the voided and PVR volumes was calculated as "bladder capacity." We also calculated as the ratio of voided volume divided by bladder capacity as "VE." Additionally, infusion volume divided by the difference between threshold and baseline pressures was calculated as "bladder compliance." In this study, 12 BOO and 12 sham rats were used for awake continuous CMG without intravesical drug administration as assessment of baseline bladder activity. In addition, 18 BOO and 12 sham rats underwent intravesical drug administration during CMG, as described below.

2.3 | Intravesical Application of Adenosine Receptor-Acting Drugs in BOO and Sham Rats

For adenosine receptor modulation, CCPA (adenosine A1 receptor agonist; Tocris Bioscience, Minneapolis, USA), ZM241385 (adenosine A2A receptor antagonist; Tocris Bioscience, Minneapolis, USA), or inosine (Sigma Aldrich, Saint Louis, USA) were administered through the intravesical catheter in BOO ($n = 6$, each drug) and sham rats ($n = 4$, each drug). According to previous reports [5, 6], the final concentrations of the respective agents were 4.1 μM, 15 μM, and 1 mM. Inosine was diluted to the final concentration in saline. As for CCPA and ZM241385, stock solutions were prepared in 100% DMSO, and were then diluted with saline to the final concentration. The final DMSO concentration of CCPA and ZM241385 solutions was less than 0.1%. After the initial baseline CMG recordings with saline for at least 60 min, we started the intravesical administration of each agent through the catheter. Then, after 60 min of drug infusion, we recorded CMG

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parameters of three consecutive voiding cycles during 60 min of recordings for the comparison with baseline CMG parameters during saline infusion. In the experiments to examine the drug effects, CMG was continuously recorded before and after the intravesical drug infusion without measuring VV or PVR in BOO rats because we tried to exclude the disruptive effects of catheter disconnection and bladder emptying on bladder activity during drug treatment.

2.4 | Transcript Levels of Adenosine Receptors in the Bladder Mucosa and Detrusor

To evaluate the change in transcript levels of adenosine receptors in the bladder mucosa and detrusor of male BOO rats, real-time PCR (RT-PCR) were performed. In separate groups of sham ($n = 8$) and BOO ($n = 8$) rats, the bladder dome was excised above the bladder trigone level, and used for further analyses. These animals did not undergo CMG to avoid the influence of repeated bladder contractions during CMG on molecular transcript levels in the bladder. A half of the bladder tissue was utilized for a histological analysis as described below, and another half, which was separated by microscissors into detrusor and mucosal layers, was stored at −80°C until mRNA analyses. Following total RNA extraction from the tissue using TRIzol reagent (Invitrogen, Carlsbad, California), 5 micrograms of RNA were reverse-transcribed into cDNA using Superscript II (Invitrogen). mRNA expressions were examined using an MX3000P real-time PCR system (Stratagene, La Jolla, California) in a 25 μL volume by SYBR Green PCR Master Mix9 (QIAGEN, Valencia, California). Reactions consisted of polymerase activation at 95°C for15 min, then denaturation cycled 40 times at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. We analyzed the expression of four adenosine receptor subtypes, such as $ADORA_1$ (A1 receptor), $ADORA_{2A}$ (A2A receptor), $ADORA_{2B}$ (A2B receptor), and $ADOR_{A3}$ (A3 receptor). The detail primers sequences were shown in a supplementary table (Table S1). A house-keeping gene (GAPDH) mRNA levels were used to calculate the ratio of each marker for statistical analyses.

2.5 | Histology

We used 4% buffered paraformaldehyde to fix bladder tissues, embedded in OCT compound, sectioned at 10 μm thickness, and stained with hematoxylin and eosin. The histological analysis of specimens was performed in a blinded manner to group allocation.

2.6 | Statistical Analysis

Results were presented in mean ± standard deviation (SD). We utilized unpaired t-test when evaluating CMG parameters and transcript levels of adenosine receptors between sham and BOO rats, and paired t-test was used when evaluating the effect of drug administration because parameter values were obtained before and after administration in the same animal. We used the JMP software (ver. 9; SAS Institute, Cary, NC) for statistical analyses and statistically significant differences were determined by p-values less than 0.05.

3 | Results

3.1 | Body and Bladder Weights

In the baseline study, there was no difference in the mean body weight between BOO ($n = 12$, 370 ± 23 g) and sham rats (n = 12, 360 ± 53 g, $p = 0.29$). However, the mean weight of BOO bladders (0.33 ± 0.10 g) was significantly greater ($p < 0.0001$) than that of sham bladders (0.11 ± 0.024 g).

3.2 | Baseline Awake Continuous CMG in Sham and BOO Rats

Representative CMG recordings are shown in Figure 1 and CMG parameters were summarized in Table 1. BOO rats exhibited significant increases in bladder contraction amplitudes during voiding (91 ± 23 cmH_2O vs. 40 ± 7.5 cmH_2O, $p < 0.0001$), the number of NVCs (0.91 ± 0.50/min vs. 0.11 ± 0.086/min, $p = 0.00020$), PVR (1200 ± 800 μL vs. 20 ± 29 μL, $p = 0.0004$), bladder capacity (2000 ± 820 μL vs. 860 ± 110 μL, $p = 0.0006$) compared with sham rats. In addition, VE was significantly ($p < 0.0001$) reduced in BOO rats (46 ± 24%) versus sham rats (98 ± 3.1%).

3.3 | Intravesical Drug Administration in Sham and BOO Rats

First, we confirmed that intravesical 0.1% DMSO administration did not affect CMG parameters in BOO or sham rats (data not shown). In sham rats, intravesical administration of CCPA, ZM241385 or Inosine did not induce significant effects on CMG parameters (Table S2) (p values of amplitude, PB, PT & NVC rate: 0.079, 0.99, 0.12 & 0.53 for CCPA; 0.51, 0.4, 0.61 & 0.43 for ZM241385; 0.43, 0.98, 0.21 & 0.45 for Inosine, respectively). In BOO rats, representative CMG traces are shown in Figure 2A (CCPA), Figure 2B (ZM241385), and Figure 2C (Inosine), and CMG parameters were summarized in Table 2. CCPA or inosine did not have significant effects on CMG parameters (p values of amplitude, PB, PT, NVC rate, VV, PVR, VE & compliance: 0.21, 0.16, 0.087, 0.17, 0.67, 0.34, 0.88 & 0.98 for CCPA; 0.082, 0.055, 0.50, 0.22, 0.72, 0.74, 0.92 & 0.32 for Inosine, respectively). whereas ZM241385 induced a significant reduction in the number of NVCs (0.50 ± 0.50/min, $p = 0.040$) from the pre-drug baseline value (0.90 ± 0.36/min) in BOO rats. The scatter plot of NVC rates using individual animal's data (Figure 2D) showed that all animals exhibited similar changes in bladder activity in response to each drug administration.

3.4 | Transcript Levels of Adenosine Receptor Subtypes in the Bladder

As shown in Figure 3, mRNA levels of adenosine A2A and A3 receptors in the bladder mucosa were significantly greater in

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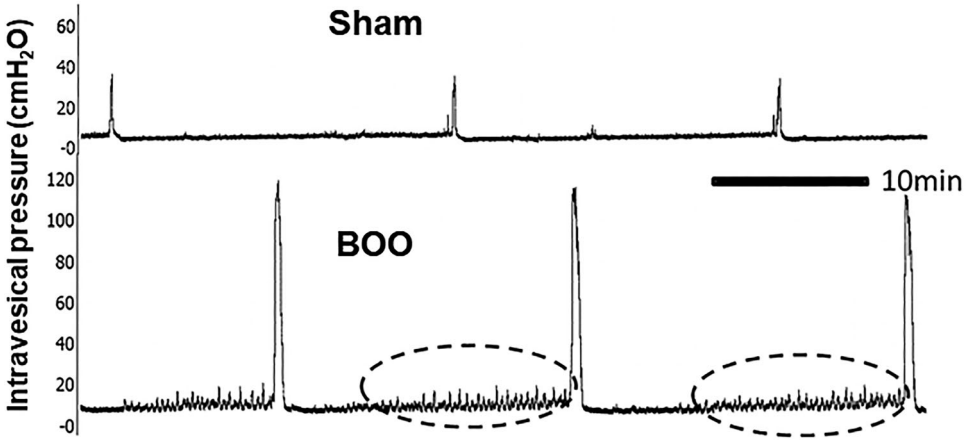

FIGURE 1 | Representative traces of baseline CMG. Comparing to sham male rats, male BOO rats (bottom trace) exhibited significant increases in bladder contraction amplitudes during voiding and the number of non-voiding contractions (NVCs) during bladder filling (dotted circle).

BOO rats than those in sham rats ($p < 0.0001$ and $p = 0.0145$, respectively). On the contrary, the mRNA level of adenosine A2B receptor was significantly decreased in the BOO bladder mucosa in BOO rats compared with sham rats ($p < 0.0001$). However, mRNA levels of adenosine receptor subtypes in the detrusor were not significantly different between sham and BOO groups (p values of adenosine A1, A2A, A2B, A3 receptors: 0.27, 0.32, 0.31 & 0.72, respectively). In addition, the expression levels of GAPDH in the bladder was not statistically different (Ct values: 21.5 ± 1.00 & 21.5 ± 0.69, respectively) in sham and BOO groups.

3.5 | Histology

As is shown in Figure 4, BOO rats revealed the thickened detrusor layer compared with sham rats whereas morphological changes in mucosal and submucosal layers were not found between sham and BOO groups.

4 | Discussion

The results of this study indicate that; (1) BOO rats had the larger-weight bladders with the thickened detrusor muscle layer than sham rats, indicative of BOO-induced bladder muscle hypertrophy, (2) BOO rats exhibited bladder overactivity evident as increased NVCs during the storage phase and voiding dysfunction evident as increased PVR, bladder contraction amplitudes and decreased VE during the urine elimination phase, compared with sham rats, (3) ZM241385, an adenosine A2A receptor antagonist, improved bladder overactivity whereas intravesical application of CCPA or inosine induced no significant effects on CMG parameters in BOO rats, (4) in the bladder mucosa, adenosine A2A and A3 receptor levels were upregulated whereas adenosine A2B receptors was down-regulated in BOO versus sham rats. Thus, it is likely that A2A adenosine receptor upregulation in the bladder contributes to bladder overactivity after 4-weeks BOO.

In the clinical medicine, storage symptoms associated with BPH often remain even after surgical removal of the enlarged prostate, and these remaining symptoms are often difficult to treat [2]. Therefore, it is important to elucidate the pathophysiology using animal models to provide the basis for the identification of new therapeutic targets of male LUTS with BPH/BOO. The animal model induced by partial urethral ligation has been used to investigate BOO-related lower urinary tract dysfunction to explore the pathophysiology of male LUTS/BPH. However, previous basic research on BOO have mostly been performed using female animals [3, 4, 9]. Thus, this study utilized male rats to produce the animal model of BOO-related male LUTS. In the baseline CMG with saline infusion into the bladder, our results indicated that male BOO rats had significant increases in the number of NVCs, the contraction amplitude during voiding, blader capacity and PVR compared with sham rats. Also, voiding efficiency (VE) showed a significant decrease in BOO rats compared with sham rats. In general, urodynamic changes of BOO rodents are not always uniform, including compensation and decompensation phases or overactive and underactive bladder conditions, which may depend on multiple factors such as the degree and duration of obstruction [10–12]. In the compensation phase, bladder overactivity evidenced as increased NVCs during the urine storage along with higher amplitudes of voiding bladder contraction is often seen whereas an underactive pattern of voiding with reduced bladder contractility and high post-void residual volume may represent the development of decompensation phase [10]. In addition, a recent study using male BOO rats demonstrated that the overactive bladder phenotype is detected in the earlier phase (4 weeks after producing BOO) whereas the underactive condition was observed in the chronic phase (16 weeks after producing BOO) in CMG recordings [13]. Based on these findings, our current study suggests that the male BOO rat model at 4 weeks, which showed the bladder overactive phenotype, could represent the early compensation phase in men with BPH, who exhibit storage LUTS such as urgency and frequent urination [10] although the possibility that there are some decompensation-like changes in 4-weeks BOO bladders cannot be excluded because of high PVR in our male BOO model. The increased bladder weight with the histological change showing detrusor muscle hypertrophy after BOO at 4 weeks also supported our interpretation that our BOO model is suitable for studying

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TABLE 1 | Baseline CMG parameters of BOO and Sham rats.

	Amplitude (cmH_2O)	PB (cmH_2O)	PT (cmH_2O)	NVC rate (NVC/min)	VV (μL)	PVR (μL)	Capacity (μL)	VE (%)	Compliance (mL/cmH_2O)
BOO ($n = 12$)	91 ± 23	4.7 ± 1.1	7.4 ± 1.5	0.91 ± 0.50	820 ± 420	1200 ± 800	2000 ± 820	46 ± 24	0.40 ± 0.23
Sham ($n = 12$)	40 ± 7.5	4.7 ± 1.3	7.9 ± 1.5	0.11 ± 0.086	840 ± 110	20 ± 29	860 ± 110	98 ± 3.1	0.29 ± 0.10
p	< 0.0001	0.98	0.46	0.00020	0.86	0.0004	0.0006	< 0.0001	0.13

lower urinary tract dysfunction at the compensation phase of BPH/BOO [10, 13].

In this study, we also revealed for the first time that the changes in the adenosine receptor system in the bladder contribute to the bladder overactive condition seen in male rats with 4-weeks BOO. The present study revealed that mRNA levels of adenosine A2A and A3 receptors in the bladder mucosa had a significant increase in BOO rats compared with sham rats whereas the expression level of adenosine A2B receptor in the bladder mucosa was significantly lower in BOO rats than that in sham rats. Furthermore, in BOO rats, intravesical CCPA (adenosine A1 receptor agonist), or inosine application did not have any effects on bladder function; however, intravesical ZM241385 (adenosine A2A receptor antagonist) application induced a significant reduction in the number of NVCs, indicating that adenosine A2A receptor inhibition has a therapeutic effect on BOO-induced bladder overactivity at the early compensation phase.

There have been previous reports on studies using animal models regarding the functional roles of adenosine receptor subtypes in the control of bladder function. Pakzad et al. showed using muscle strips obtained from human and guinea pig bladders that adenosine or an A1/A2 receptor agonist (NECA) elicited reductions in carbachol-induced and nerve-mediated contractions, which were antagonized by an A2B receptor antagonist (alloxazine), but not an A2A antagonist (ZM-241385) [14]. Additionally, an A1 receptor agonist (CCPA) reduced nerve-mediated muscle strip contractions of human bladders with neurogenic detrusor overactivity and guinea pig bladder muscles strips [14]. In other studies using female rats, it has been shown that systemic, brain or intrathecal activation of adenosine A1 receptors inhibited the micturition reflex [15, 16] and that adenosine A2A receptor-mediated excitatory effects were enhanced at the spinal cord level following cystitis, in which bladder overactivity was induced by C-fiber bladder afferent stimulation [15]. In addition, it has been revealed that inhibition of adenosine A2A receptors in bladder afferent pathways reduced bladder overactivity in rats with cyclophosphamide-induced cystitis [17]. Taking together, it seems likely that adenosine A1 and A2A receptors expressed in neural pathways (both central and peripheral) innervating the bladder play inhibitory and excitatory roles in the control of micturition reflexes, respectively, whereas adenosine A2B receptors, but not A2A receptors, is involved in the inhibitory control of detrusor muscle contractility under the normal condition. However, the pathophysiological role of adenosine receptor mechanisms in lower urinary tract dysfunction induced by BOO has not previously been investigated.

Thus, this is the first study to demonstrate that adenosine A2A receptors expressed in the bladder, which are minimally involved in the control of normal bladder activity [14], are up-regulated in the bladder mucosa that includes the urothelium and suburothelial lamina propria, and contribute to bladder overactivity evident as increased NVCs after BOO. Therefore, we propose that modulating the adenosine receptor signaling may be an attractive therapeutic option in male LUTS/BOO patients with bladder overactivity. In addition, because adenosine A2B receptors expressed in the bladder are reportedly

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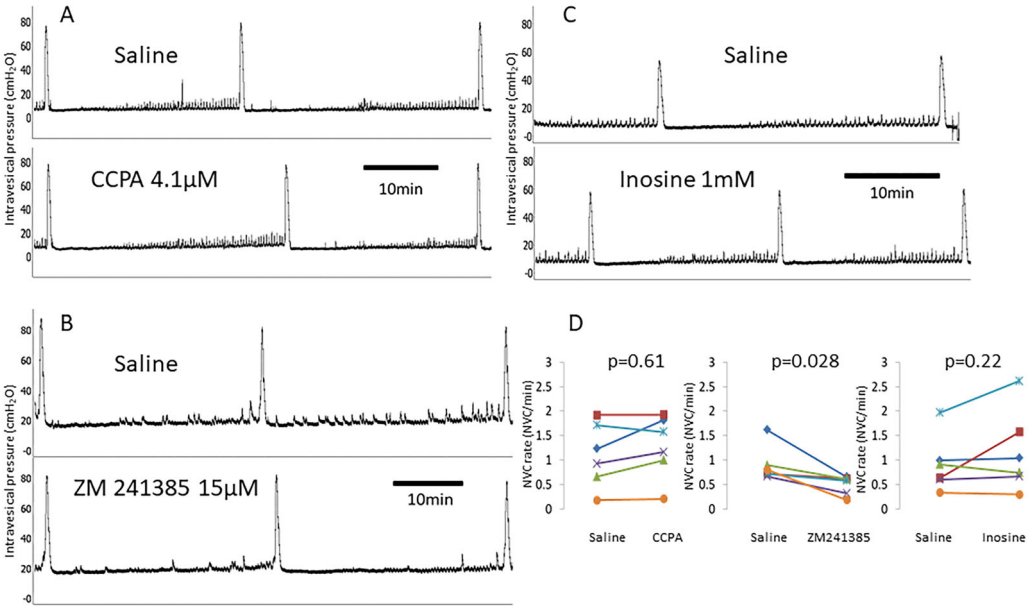

FIGURE 2 | Representative CMG traces of male BOO rats with intravesical drug administration of CCPA (A), ZM241385 (B), or Inosine (C). In each figure, the upper and lower traces showed CMG recordings during saline instillation and after intravesical drug treatment, respectively. CCPA or inosine did not have any effects on CMG parameters; however, ZM241385 significantly decreased the number of non-voiding contractions (NVCs). The scatter plot of NVC rates using individual animal's data (D) showed that all animals had similar changes in bladder activity in response to each drug administration.

TABLE 2 | CMG parameters with intravesical drug administration in BOO rats.

	Amplitude (cmH_2O)	PB (cmH_2O)	PT (cmH_2O)	NVC rate (NVC/min)	VV (μL)	PVR (μL)	VE (%)	Compliance (mL/cmH_2O)
saline	68 ± 27	6.8 ± 2.6	10 ± 2.9	1.1 ± 0.65	1500 ± 1100	710 ± 580	70 ± 19	0.44 ± 0.12
CCPA	65 ± 28	7.7 ± 1.8	12 ± 2.1	1.3 ± 0.64	1400 ± 640	800 ± 750	70 ± 26	0.44 ± 0.11
p	0.21	0.16	0.087	0.17	0.67	0.34	0.88	0.98
saline	72 ± 28	7.7 ± 4.2	11 ± 4.2	0.90 ± 0.36	1100 ± 240	280 ± 250	80 ± 17	0.45 ± 0.27
ZM241385	74 ± 44	7.9 ± 4.5	11 ± 4.1	0.50 ± 0.50	1300 ± 520	240 ± 290	84 ± 17	0.52 ± 0.20
p	0.71	0.60	0.28	0.040	0.63	0.19	0.24	0.14
saline	74 ± 30	4.4 ± 1.3	7.0 ± 0.82	0.91 ± 0.57	910 ± 550	890 ± 780	55 ± 26	0.46 ± 0.30
Inosine	69 ± 25	5.0 ± 1.3	7.4 ± 1.6	1.2 ± 0.83	870 ± 490	850 ± 670	54 ± 20	0.64 ± 0.47
p	0.082	0.055	0.50	0.22	0.72	0.74	0.92	0.32

inhibitory to reduce bladder contractility when activated [14], our present results showing the decreased expression level of A2B receptors in the bladder mucosa in male BOO rats suggest the possibility that the reduced inhibitory effects of A2B receptors also contribute to BOO-induced bladder overactivity although further studies are needed to clarify this point.

There are some limitations in this study. First, we evaluated lower urinary tract dysfunction only in the early phase of BOO. As described above, the long -term BOO causes bladder decompensation often associated with the underactive bladder condition. However, the role and distribution of adenosine receptors in underactive bladder have not been investigated so far in BOO or other animal models. Secondly, we used different bladder infusion speeds to compensate for the functional bladder volume, which varied in BOO rats. Thus, it is possible that different stretch velocity of the bladder wall may have affected bladder activity including BOO-induced increases in NVC, for example, due to the higher amount of stretch-mediated release of ATP, the adenosine precursor, in the bladder. Nevertheless, in this study, the inhibitory effects of an adenosine A2A receptor antagonist on NVCs were consistently found in BOO rats (Figure 2D), suggesting that the different infusion speed did not affect the results and conclusion regarding the A2A-receptor-mediated mechanism of BOO-induced bladder overactivity. Thirdly, previous studies showed that BOO-induced bladder decompensation stage is often associated with bladder wall remodeling characterized by tissue fibrosis [10, 13]. However, it is unclear whether changes in the adenosine receptor mechanism in the bladder are involved in bladder wall remodeling following long-term BOO. Thus, future studies are needed to examine the

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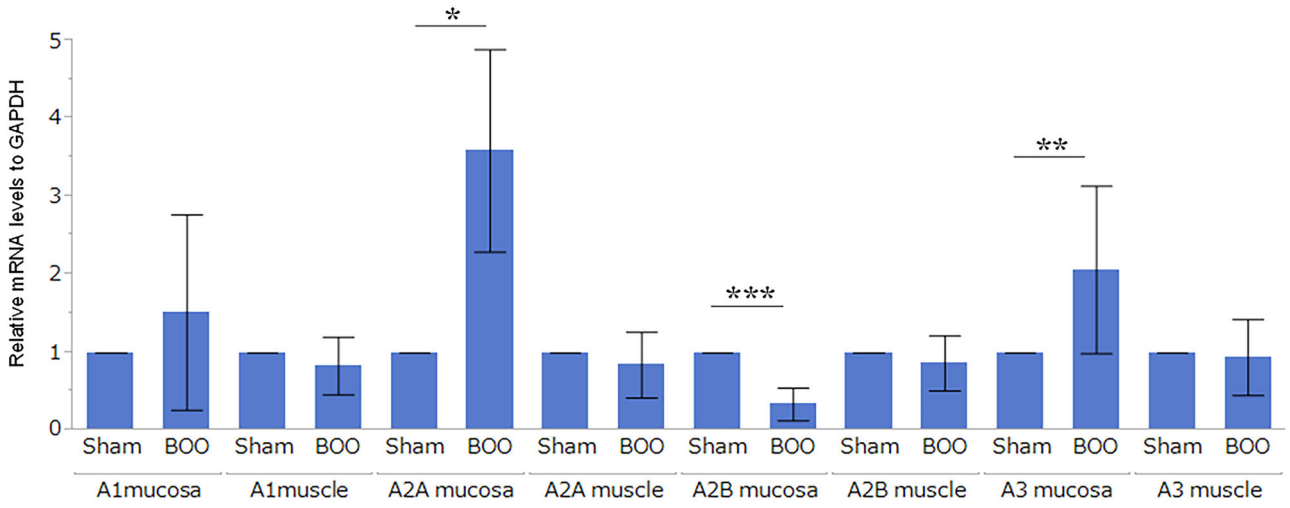

FIGURE 3 | Transcript levels of adenosine receptors in the bladder mucosa and detrusor. mRNA expression levels of adenosine A2A receptors and A3 receptors in the bladder mucosa were significantly increased in BOO rats compared with sham rats ($p^* < 0.0001$ and $p^{**} = 0.0145$, respectively). mRNA expression level of adenosine A2B receptor in the bladder mucosa was significantly decreased in BOO rats compared with sham rats ($p^{***} < 0.0001$).

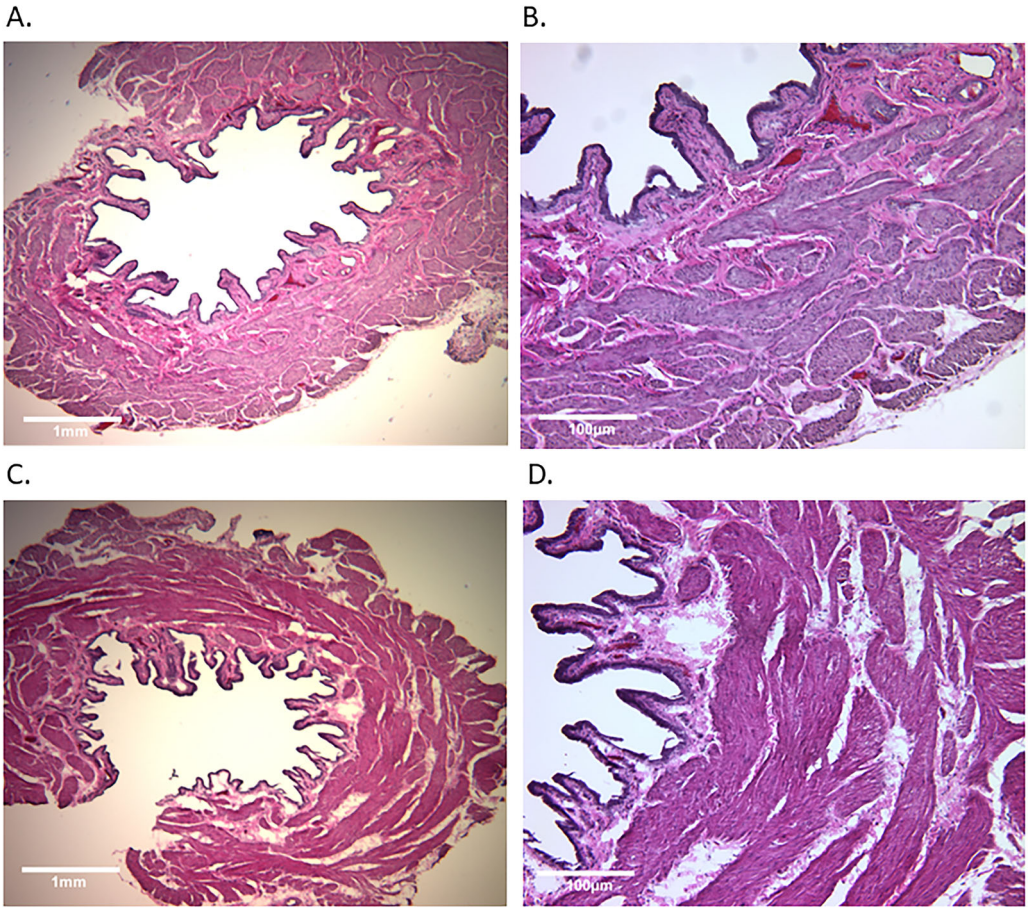

FIGURE 4 | Photomicrographs of bladder sections from sham (A, B) and BOO rats (C, D). The BOO rat bladder showed the thickened detrusor layer compared with sham rats (B vs. D), whereas there is no remarkable changes in mucosal and submucosal layers between BOO and sham rats.

long-term effects of BOO on adenosine receptor functions leading to underactive bladder and/or tissue remodeling in the BOO-induced decompensation phase. Lastly, although we have found in this study that the expression level of adenosine A3 receptors in the bladder mucosa was increased in BOO rats, little is known about the role of A3 receptors in the control of micturition reflexes. We will plan future studies to investigate the adenosine A3 mechanisms in lower urinary tract dysfunction under normal and pathological conditions including BOO.

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5 | Conclusions

In conclusion, the male rat model of 4-weeks BOO seems to be suitable to study the pathophysiology of bladder overactivity during the compensation phase of BOO. Also, targeting therapies of the adenosine A2A receptor subtype, which is upregulated in the bladder after BOO, may be effective for the treatment of male BPH/LUTS patients with OAB.

Author Contributions

Conceptualization: Paul Watton, Anne M. Robertson, Naoki Yoshimura; Data curation: Ei-ichiro Takaoka, Masahiro Kurobe, Kanako Matsuoka; Formal analysis: Kanako Matsuoka, Tadanobu Kamijo, Shingo Kimura; Funding acquisition: Paul Watton, Anne M. Robertson, Naoki Yoshimura; Investigation: Ei-ichiro Takaoka, Masahiro Kurobe, Kanako Matsuoka; Methodology: Naoki Yoshimura, Kanako Matsuoka, Shingo Kimura; Project administration: Anne M. Robertson, Naoki Yoshimura; Resources: Ei-ichiro Takaoka, Naoki Yoshimura; Software: Ei-ichiro Takaoka; Supervision: Naoki Yoshimura; Validation: Paul Watton, Anne M. Robertson, Naoki Yoshimura; Writing – original draft: Ei-ichiro Takaoka; Writing – review & editing: Naoki Yoshimura, Anne M. Robertson.

Ethics Statement

All animal experiments were conducted in accordance with the ARRIVE and NIH guidelines, and were approved by the University of Pittsburgh IACUC (#22122280).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

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